HYBRIDIZATION EXPERIMENTS WITH AN ALL–FEMALE FISH OF THE GENUS POECILIOPSIS [1]

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The sex-determining mechanism of the monosexual strains of *Poeciliopsis* is unique to vertebrate animals. Two such all-female populations have thus far been found among the 16 species that form this principally Mexican genus of poeciliid fishes (Miller and Schultz, 1959). Each of these all-female strains, *P.* Cx and *P.* Fx, is superimposed on a bisexual or normal species (*P.* C, now described as *P. lucida* by Miller, 1960, and *P.* F, still undescribed) and is dependent on the male of that species for fertilization.

Laboratory studies (Schultz, 1961) have provided the following hypothesis regarding the origin and perpetuation of monosexuality in *Poeciliopsis:*

1. Hybrid crosses between bisexual species indicate that the sex-determining mechanism varies in strength between different members of the genus; thus when a male from a species with a "strong" mechanism is mated to a female of a species with a "weak" mechanism, the progeny are predominantly male. From the reciprocal cross, however, predominantly females are produced.
2. It is believed that all-female "species" of *Poeciliopsis* originated in nature when a very "strong" female crossed with a "weak" male and so produced all female hybrid offspring. These now maintain themselves by continuously backcrossing with the weak male.
3. In a series of backcrosses one might expect such a mechanism to break down rapidly and to re-establish normal sex ratios—provided random segregation of chromosomes takes place. By using a marker gene, it was established that at least one chromosome is not transmitted through the egg; and it is possible that a large number or even all of the paternal chromosomes of each generation are lost during oogenesis. If the latter obtains, then, the ova receive only the maternal complement of chromosomes generation after generation, even though the all-female progeny do reflect characteristics of the father of each mating.

Exclusion of paternal traits from the eggs of *Poeciliopsis* Cx seems to be the female version of a similar mechanism for chromosome elimination in *Sciara* (fungus gnats), wherein all paternal chromosomes are pinched off in a tiny bud during spermatogenesis (Swanson, 1957).

The mode of inheritance of the monosexual *Poeciliopsis* should not be confused with that of *Poecilia* (*Mollienesia*) *formosa,* in which all-femaleness results from gynogenesis. In *P. formosa* the sperm of other species is essential to activate

development of the ova but there is no gametic fusion; therefore, the progeny are identical to the mother (Hubbs and Hubbs, 1932, 1946; Kallman, 1962).

If monosexuality in *Poeciliopsis* has evolved in the manner postulated, one might expect to find among the 16 bisexual species some that have sex-determining mechanisms sufficiently strong to counter-balance the all-femaleness. In previous studies it was demonstrated that *P.* Fx would hybridize with the allopatric *P. lucida* and that *P.* Cx would hybridize with the *P.* F.; but from both matings, exclusively female progeny were obtained. *P. lucida* and *P.* F are closely related; and, although mating tests demonstrate that there is a difference in the sex-determining potential of the two species, neither apparently has sufficient strength to topple the all-female mechanism.

Living in the Rio Mocorito with *lucida* and Cx is *P. latidens* Garman, a species that differs considerably from the mates of the all-female forms. This species when mated to Cx produces progeny of both sexes. All of the male progeny thus far have been sterile but the females are fertile.

In the 1961 study, only a single marker gene was available to trace the route of paternal chromosomes in matings with monosexual forms; and although inferences could be made, based on the consistency of maternal inheritance, it could not be demonstrated with certainty that any more than one paternally derived chromosome was consistently lost at the time the F_1 ova were produced. The great number of characters by which *latidens* differs from Cx and *lucida* provides the necessary tools for determining to what extent paternal chromosomes are transmitted through the ova in various hybrid matings.

MATERIALS AND METHODS

All of the specimens used in this study were descendants of fishes collected from the Rio Mocorito in the state of Sinoloa, Mexico, near the villages of San Benito and La Huerte by Miller and Schultz, 1959; Miller and Huddle, 1961; and Miller and Schultz, 1963. Gravid females were transported to the laboratory by air freight and upon arrival were isolated in two- and four-gallon aquaria. The young born to these wild females provided the progeny used in the mating experiments. Offspring produced from the various matings were all reared to approximately the same size (33 to 35 mm.) to facilitate quantitative comparisons. These were killed and fixed in 7% formalin and stored in 70% alcohol. The lower jaws were later removed, cleared in potassium hydroxide and stained in alizarin for osteological studies. Tooth measurements were made with an eyepiece micrometer. All quantitative comparisons were based on the t-test using 5% as the level of significance.

RESULTS

Sex ratios

Over the 5 years that Rio Mocorito fishes were maintained in the laboratory, 10 generations of *Poeciliopsis* Cx were produced from matings with *P. lucida* males. Of the 306 Cx young reared to maturity, not one was a male (Table I). There was no evidence of an unbalanced sex ratio in either of the two bisexual species involved in the study: (1) 44 *lucida* pairs in 13 generations produced 366 females

and 376 males (1:1 ratio $\chi^2 = 0.136$) and (2) 11 *latidens* pairs in 5 generations produced 60 females and 52 males (1:1 ratio, $\chi^2 = 0.067$).

In the hybrid cross Cx × *latidens*, 6 pairs produced 87 F_1 progeny. Here, for the first time in the laboratory males were born to Cx mothers. The sex ratio, however, was far from 1:1 since 81 of these were females and only 6 were males. All males were produced from two females (5 from one and one from another) in spite of the fact that the non-male producers had substantial numbers of offspring.

TABLE I

Sex ratios of stock cultures and hybrid crosses

	Number of pairs involved	Offspring produced			Number of generations represented
		Females	Males	Total	
lucida × lucida	44	366	376	742	13
latidens × latidens	11	60	52	112	5
Cx × lucida	26	306	0	306	10
Cx × latidens	6	81	6	87	—
(Cx × latidens) × latidens	3	18	0	18	—
(Cx × latidens) × lucida	2	8	0	8	—

Backcrosses of the F_1 female to the *latidens* male were successful three times for a total of 18 progeny, all of which were females. Two matings back to the *lucida* male resulted in 8 offspring which were again all females.

Genitalia

Classification of poeciliid fishes depends largely upon the morphology of the male's copulatory organ, known as a gonopodium. This structure derives from a modification of the anal fin. Elongation and specialization of the fin rays of juvenile males in *Poeciliopsis* leads to the formation of an imperfectly closed tube—imperfect in that a narrow groove remains open along the left side. At the tip beyond the termination of the fin rays, there is a fleshy protrusion of the cutis. This structure varies in shape from one species to the next and probably serves as part of the holdfast mechanism during copulation. Rosen and Bailey (1963) in revising the family Poeciliidae emphasized the importance of the gonopodium and its internal skeletal supports, the suspensorium, in the systematics of the group. Although a comparison of suspensoria within the genus *Poeciliopsis* is yet to be worked out, there is little question that the tip of the gonopodium provides a useful taxonomic tool (Hubbs and Miller, 1954; Miller, 1960).

The genitalia of females, in contrast to males, has played an insignificant role in the classification of poeciliid fishes in general. In *Poeciliopsis*, however, the shape of the genital papillus and the distribution of melanin in this region are of considerable value for the separation of species. Extending from the base of the anus to the origin of the anal fin is a scaleless depression of soft tissue, the genital pit. The fleshy papillus with the genital opening at its tip arises from this pit as a more or less cone-shaped structure. All forms of *Poeciliopsis* dealt with in this
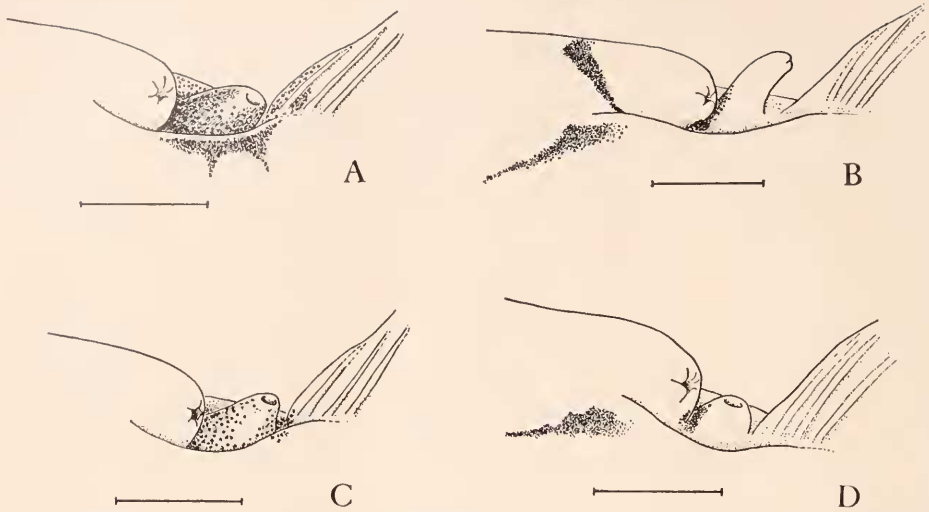
FIGURE 1.   Genital region of *Poeciliopsis* females: A, *P. latidens;* B, *P. lucida;* C, Cx × *latidens*
or Cx × *latidens* × *latidens;* and D, Cx or Cx × *latidens* × *lucida.*

study can be easily identified on the basis of the female's genitalia, a factor of no
small importance in a group containing all-female fishes.

    P. latidens.   The genital pit and papillus of this species are so densely covered
with melanin that the individual melanophores can be seen only in the peripheral
region.   This blackened area in *latidens* is more extensive than in *P. lucida,* Cx
or the Cx × *latidens* hybrids (Fig. 1).   It forms a large blotch that overflows the
pit and extends laterally across the first row of scales and posteriorly on to the anal
fin as far as the second or third fin ray and distally on the anal fin one-half to
three-fourths the length of the first ray.   Extending forward along both sides of
the anal elevation are two horns of pigment that lie deep in the tissues.   These are
not well developed in live *latidens* females; in preserved specimens, they are usually
not seen at all because of the opacity of the over-lying tissues.   The papillus in live
females resembles a squat volcano that practically fills the genital pit.   Upon preser-
vation, however, it becomes more slender, often flexed with the genital opening
drawn nearly closed.

    The structure of the gonopodium of *latidens* has been depicted in detail by Hubbs
and Miller (1954).   It is sufficient here to consider only the tip of the organ which
hooks strongly downward, so that, viewed laterally it resembles the letter "J,"
fattened at the bottom by an unsegmented fleshy portion and supported on the
inner curvature by the tapered fin rays (Fig. 2).

    P. lucida.   The genital papillus of *P. lucida* is a long, slender, finger-like
process (Fig. 1).   Development of melanin is minimal and restricted primarily to
the pit region between the papillus and the anus; only a few melanophores extend
on to the papillus where they are confined to the anterior surface.   Scattered
melanophores are occasionally found in the posterior part of the pit but by and
large this area is silvery-white as is most of the papillus.   Two horns of black

pigment arise from the anterior margin of the pit and course upward and forward through the tissue on both sides of the anus, forming a V-shaped pattern near the surface. Anterior to the base of the anus a curved black line crosses from one arm of the V to the other. Although all of the other characters are present in preserved specimens, the pre-anal cross-bar is quickly lost due to the opacity of the over-lying tissues.

The terminal rays of the *lucida* gonopodium are not curved as in *latidens*. There is, however, a fleshy unsegmented tip distinctive from that of *latidens* by an anterior projection described by Miller (1960) as a "retrorse hook" (Fig. 2).

P. "Cx." In this all-female form, distribution of melanin about the genital region is the same as in *lucida*, being most pronounced in the depression between the anus and the papillus, extending somewhat up the anterior side of the papillus but almost totally absent from the posterior part of the pit (Fig. 1). The all-female genitalia differs from that of *lucida* on two counts: (1) The papillus is short and fat instead of long and finger-like, and (2) the curved pre-anal cross-bar is lacking, though a pre-genital V does extend upward and forward along the sides of the anus.

Cx × *latidens* and Cx × *latidens* × *latidens*. No difference could be found between the genitalia of the F_1 and the backcross hybrids; hence, they are described as
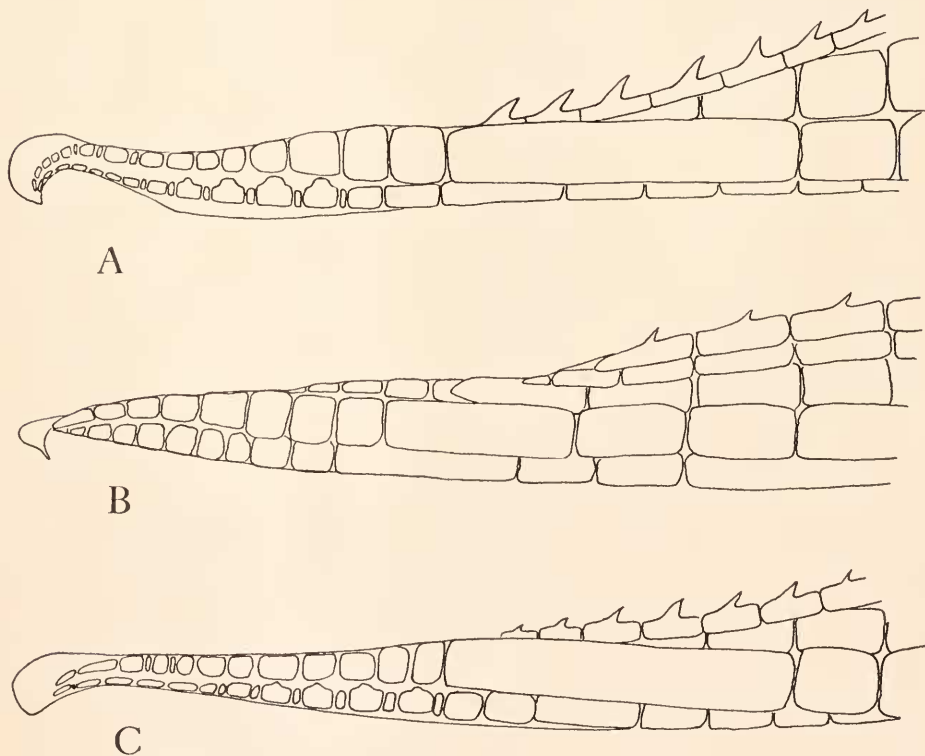

FIGURE 2. Gonopodial tips of (A) *Poeciliopsis latidens,* (B) *P. lucida,* and (C) Cx × *latidens* F_1 hybrid.

one (Fig. 1). The distribution of melanin varies among individuals but there is no problem distinguishing the hybrids from either Cx or *latidens*. At maximum development melanin is found scattered over most of the papillus and throughout the pit with some pigment sprinkled over the edge of the pit. A few melanophores dot the base of the anal fin near the pit but do not extend down the fin as they do in *latidens*. In the most densely pigmented individuals, the blackened area is not only less extensive than in *latidens* but the melanophores are more distinct and do not present a uniformly black coating. At minimal development the papillus is without melanin but unlike Cx or *lucida* there is always a sprinkling of melanophores in the posterior part of the pit. The shape of the papillus is not consistently different from that of *latidens*.

The tips of the gonopodia of the few hybrid males produced are less curved than those of *latidens* and lack the retrorse hook of *lucida*. The terminal rays curve only slightly and the fleshy tip viewed from the side curves more gently than in either *lucida* or *latidens*.

Mouthparts—lips, jaws, and teeth

The jaw bones, teeth and lips of *Poeciliopsis* prove to be among the most diagnostic characters available for separating members of the genus. As a matter of convenience, investigation was restricted to the lower jaw which can be easily removed, cleared, stained and examined under the microscope without damaging the rest of the specimen.

The teeth are arranged in two groups: (1) the large, outer teeth, and (2) the tiny, inner teeth. The outer teeth are planted firmly in the middle of the anterior face of the dentary bone. They curve upward over the face of this bone and turn sharply inward as they emerge from the flesh into the mouth. The shank of the outer tooth is rounded in cross-section and completely embedded in the tissues of the mouth. Most of the flattened portion or blade is also embedded with only the tip exposed. The shape and width of the blade vary from one species to the next. Behind the single row of outer teeth are one to many irregular rows of the tiny, inner teeth embedded on the dorsal surface of the dentaries where they run along the anterior margin and extend a short way down the outer margin of each jaw.

When viewed from above the cleared and stained dentary bone (Fig. 3) resolves itself into three prominences: (1) A large flat shelf upon which the inner teeth are secured. This thins out posteriorly to form an over-hanging margin with a contour that is species-specific. (2) There is a posterior wing forming the sides of the jaw; it has one groove on the outside for muscle attachment and one on the inside that receives the articular bone, and (3) an elbow projects from beneath the middle of the shelf and extends posteriorly and downward a short distance before making a right-angle mesial turn, terminating short of the midline.

The lips of some species are thick and rounded in cross-section but in others they are thin and flap-like. From a dorsal view they may be strongly curved, providing a streamlined snout, or they may run straight across, giving a squared-off appearance. The lower lip precedes the upper as is characteristic of other surface feeders.

P. lucida. The dentary bone of *P. lucida* forms a right angle at the anterior-
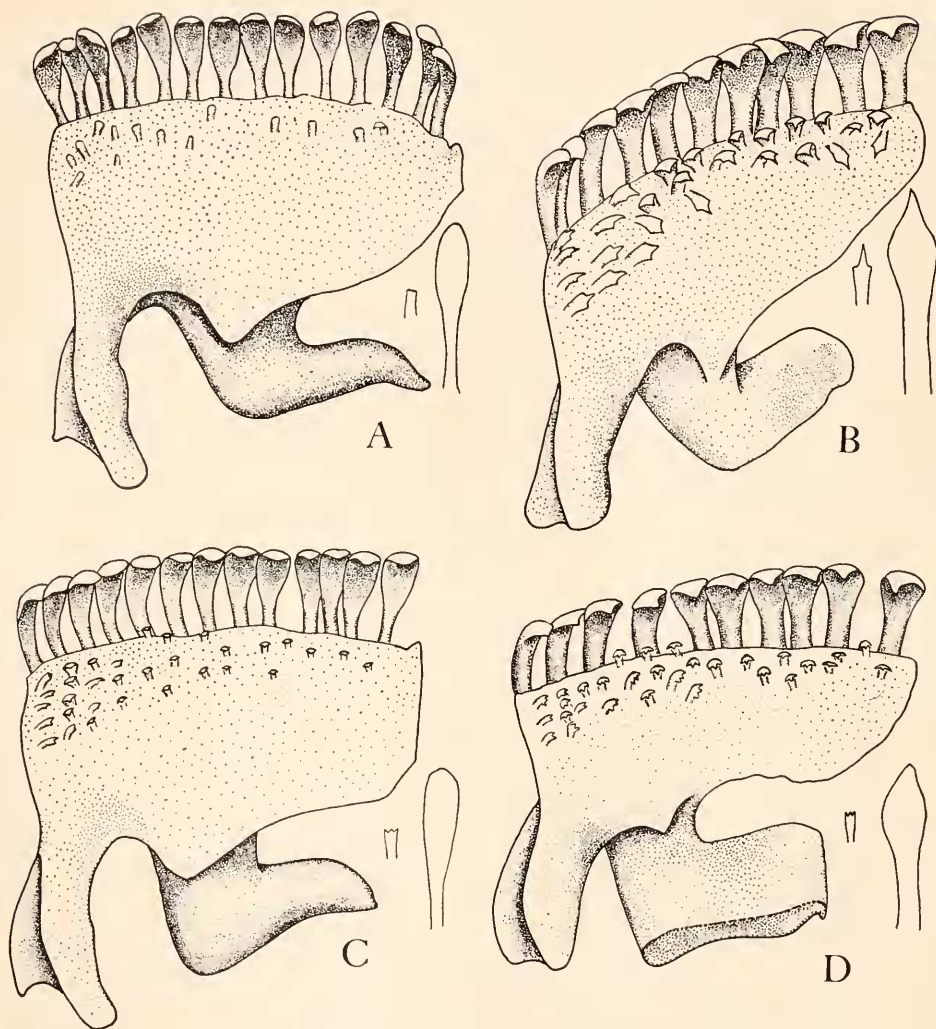
FIGURE 3. Left dentary bone of (A) *Poeciliopsis lucida*, (B) *P. latidens*, (C) Cx, and (D) Cx × *latidens* F₁ hybrid.

lateral corner; but as the anterior margin runs toward the midline it curves inward producing an indentation at the symphysis of the two dentary bones. The shelf that supports the inner teeth and forms the floor of the lower jaw is well developed and extends backward in a broadly curving, posterior-mesial over-hanging margin. The elbow, after making its right-angle turn, is rounded in cross-section and tapers toward the tip. The thin, straight lip-line is interrupted only by a deep median indentation.

The outer teeth are smaller (53.4 microns wide, Table II) and more numerous (average per lower jaw 29.07, Table III) than in any of the other forms of

TABLE II

Width in microns of outer and inner teeth. Horizontal lines separate groups significantly different ($p < 0.05$) from each other

Mating	Average maximum width of inner teeth	Average maximum width of outer teeth	Average minimum width of outer teeth
lucida × lucida	19.4 ± 0.46	53.4 ± 0.92	18.6 ± 1.8
Cx × lucida	24.2 ± 1.6	58.4 ± 0.6	21.6 ± 0.2
Cx × latidens × lucida	23.8 ± 0.8	58.0 ± 0.6	21.8 ± 0.2
Cx × latidens	33.2 ± 3.2	80.8 ± 4.2	40.8 ± 0.6
Cx × latidens × latidens	33.4 ± 0.5	79.4 ± 4.6	41.8 ± 0.8
latidens × latidens	37.2 ± 1.2	91.0 ± 1.4	44.8 ± 0.6

Poeciliopsis considered here. The tips of these teeth are mostly rounded or spatula-shaped; some form a slanted chisel but none are pointed or spear-shaped. The flattened portion or blade is extensive and about two and one-half times as wide as the short rounded shank. Not only are the inner teeth the smallest (19.4 microns) but they are also the least numerous of any of the forms studied (35.36). They are in the main poorly developed, being mostly peg-shaped and spatula-shaped with fewer than one-fourth of them suggesting the tricuspid condition.

P. latidens. The snout of this species is strongly curved. It has thick, full lips with no suggestion of a median indentation. Curvature of the snout stems not only from the fleshy elements but also from the skeletal structure. From the point of symphysis of the dentary bones the anterior margin sweeps back in a curve forming a 120° angle with the lateral margins—this in contrast to *lucida* and Cx, wherein the angle is 90°. The dorsal plate of the dentary does not have a well developed over-hang; rather, the posterior margin cuts diagonally up and across from the base of the elbow, eliminating the medial margin except for a narrow symphysis. In cross-section the terminal arm of the elbow is square.

The outer teeth are fewer (24.15) than in Cx or *lucida* but they are larger (91.0 microns). There is a heavy, long shank embedded in the flesh with a short spear-shaped blade. The inner teeth are large (37.2 microns) and well developed. These are tricuspid with the middle tine longer than the two outer tines. Often

TABLE III

Mean number of inner and outer teeth in lower jaw. Horizontal lines separate groups significantly different ($p < 0.05$) from each other

Mating	N	Outer	Inner
lucida × lucida	14	29.07 ± 0.71	35.36 ± 1.16
Cx × lucida	14	27.00 ± 0.47	66.13 ± 3.59
Cx × latidens × lucida	7	27.29 ± 0.35	61.14 ± 3.35
Cx × latidens	14	21.47 ± 0.46	64.33 ± 3.80
Cx × latidens × latidens	7	20.88 ± 0.56	76.14 ± 2.86
latidens × latidens	14	24.15 ± 0.67	60.54 ± 4.81

the outer tines are worn down to little more than a shoulder. There are nearly twice as many inner teeth (66.13) as in *lucida* but the average number is not significantly different from the rest of the forms considered here.

P. C.r. The shape of the lips and dentary bones of Cx resembles those of *lucida* in that their anterior-lateral margins form a square-cornered mouth. Except for a median indentation, somewhat less pronounced than in *lucida,* the lips and anterior margin of the dentaries run straight across. Posteriorly the dentary plate is even better developed than in *lucida.* The posterior over-hang rather than being rounded is squared off; thus the dentary plate is nearly rectangular. The elbow is rounded and tapers toward the tip.

The outer teeth do not differ greatly in shape from those of *lucida.* They are spatula to chisel-shaped, slightly wider (58.4 microns) and on the average slightly fewer (27.0). A short, slender peduncle-like shank and long blade are characteristic of both forms.

It is in the inner teeth that the primary differences are to be noted. In contrast to *lucida,* wherein most of the inner teeth are unicuspid, those of Cx are basically tricuspid. Fewer than one-fourth are of the *lucida* type, even though the specimens examined were sired by *lucida* males. The tricuspid condition here differs from that characteristic of *latidens* in that the middle tine is not greatly elongated; furthermore, these teeth are only about two-thirds the size of the *latidens* teeth (24.2 *vs.* 37.2 microns). The number of inner teeth in the lower jaw of Cx is nearly twice that of *lucida* (66.13 *vs.* 35.36) but not significantly different from *latidens* (60.54). These are arranged in two or three uneven rows along the anterior margin of the dentaries with some clustering along the lateral margins.

P. Cx × *P. latidens.* The snout of the Cx × *latidens* hybrid is rounded but less so than that of *latidens.* The moderately thickened lips show either no evidence of a median indentation or in some only a trace. Each of the dentary bones is slightly rounded both laterally and medially; there is, thus, a slight indentation at the symphysis, even though there is little reflection of this trait in the thick lips. The angle formed by the anterior-lateral margins of the dentaries is less pronounced (100°) than that of *P. latidens* (120°). In the hybrid, the shelf is narrow as in *latidens* but the posterior over-hang is better developed and has a rounded corner at the medial margin rather than a diagonal cut straight to the symphysis. The elbow, unlike that of *lucida,* Cx, or *latidens,* is broad and flattened.

The outer teeth of the hybrid resemble those of *latidens:* they have a thickset shank that comprises more of the tooth length than does the blade and there is a spear-shaped tip which is, however, less pointed than in *latidens.* The number of outer teeth in the lower jaw (21.47) is less than that of either parent (Cx—27.00 and *latidens*—24.15); whereas the width (80.8 microns), although intermediate (58.4 and 91.0 microns), lies closer to the dimensions of the wider *latidens* teeth.

The inner teeth are all tricuspid; and although their size (33.2 microns) more closely approximates that of *latidens* (37.2 microns), they lack the long middle tine and, hence, are shaped more like the smaller (24.2 microns) Cx type. Their average number per jaw (64.33) does not differ significantly from that of Cx or *latidens.*

Cx × *latidens* × *latidens.* The shape of the lips, dentary bones and teeth of the backcross progeny is identical to that of the F_1 and, therefore, need not be

repeated. No significant difference was found in either the tooth numbers or their dimensions.

Cx × *latidens* × *lucida*. The mouth parts of progeny from this mating (Tables II and III) are identical to those of Cx.

Ova size

The diameters of ova from *latidens*, *Cx*, and *lucida* females are distinctly different: 2.22, 1.81, and 1.53 mm., respectively (Table IV). Progeny of the Cx × *latidens* and Cx × *latidens* × *latidens* crosses had ova sizes identical to those of Cx. In spite of the fact that *latidens* has much larger ova, no increase in size resulted through hybridization with this species.

TABLE IV

Ova sizes (mm.) of progeny from various matings. Horizontal lines separate groups significantly different ($P < .05$) from each other

Mating	N	Mean
latidens × latidens	15	2.22 ± 0.03
lucida × lucida	15	1.53 ± 0.02
Cx × lucida	15	1.81 ± 0.08
Cx × latidens	15	1.84 ± 0.06
Cx × latidens × lucida	15	1.78 ± 0.08
Cx × latidens × latidens	15	1.77 ± 0.02

Inheritance of body pigment pattern

The most prominent feature of *P. latidens* is the pattern of black vertical bars and spots along the sides of the body. Spots, 8 to 11 per side, vary from 0.25 mm. to 1.25 mm. in diameter and are arranged along the midline. The bars are 0.5 to 1 mm. wide and extend from the back down the sides where they always pass through a spot and extend a short distance below the midline. The number of bars varies from one to five per side in *latidens* from the Mocorito, but three or four is by far the most common. In other rivers the mode may shift to either side of this. The first bar in practically all specimens is located just back of the pectoral fins. No other consistency is evident in the arrangement of spots and bars among individuals or even between the left and right sides. Bars may alternate with spots or as many as four bars may occur in succession uninterrupted by spots.

This pattern of bars and spots has been test mated (unpublished data) against the narrow barred pattern of *P. fasciata* (Meek), a species closely related to *latidens,* and found to have polygenic inheritance, in that there is a blending in the F_1 generation and a gradual restoration of the parental phenotype in the backcrosses.

P. lucida and Cx are uniformly olive color with no bars and spots. Mating combinations of *lucida* and Cx with the boldly marked *latidens* thus involve phenotypes of high contrast. When the plain or unmarked Cx was mated to the barred and spotted *latidens,* all 81 F_1 offspring had spots but no bars (Fig. 4). The spots of the hybrid were similar to those of *latidens* as regards size and position but were

in general fewer in number, the range being one to 7 per side with no number favored over any other. The 6 males resulting from this mating exhibited the same spotted pattern found in the 81 females.

In the backcross to *latidens*, one might expect with normal segregation to find a few bars added to the spotted pattern or an elongation of the spots as was characteristic of the *latidens* × *fasciata* crosses; however, this was not the case, the pattern remained unaltered; the spotted condition of the 18 backcross progeny was identical to that of the F₁ generation. One might further expect that the
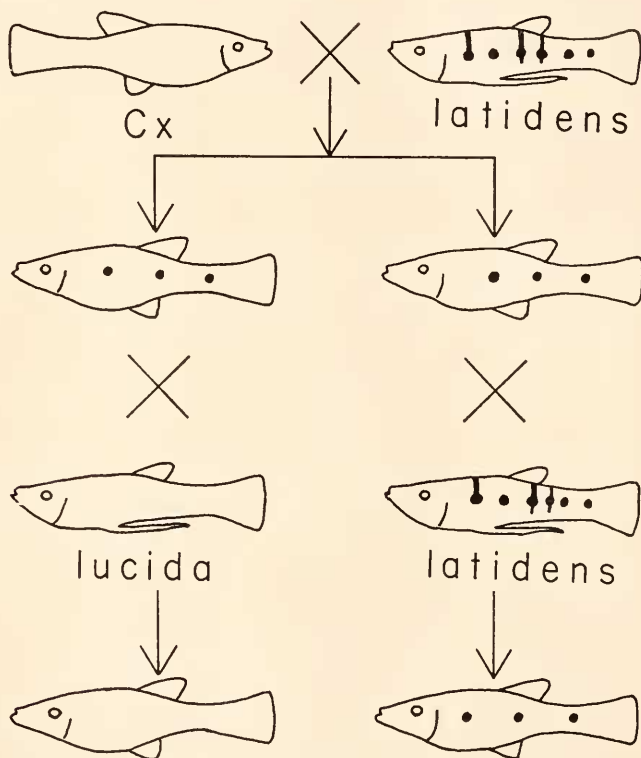

Figure 4. Inheritance of body pigment pattern in hybrid crosses.

Cx × *latidens* F₁ offspring mated to *lucida* would bring about a reduction in the number of spots or perhaps a dilution in the intensity of expression. The results, however, were more dramatic in that spots were totally lacking in the 8 progeny from this mating.

Discussion

Of the 14 species of *Poeciliopsis* that have been studied in the laboratory, 10 have produced fertile offspring when mated with the all-female form *Poeciliopsis* Cx (Miller and Schultz, unpublished). In all of these matings the offspring shared characteristics of both parents, and must, therefore, be considered true hybrids and

not the fruit of parthenogenesis or gynogenesis. It is important from both an evolutionary and a systematic point of view to determine the impact that fertile hybrids such as these might have on a natural population. The question in point is whether or not characteristics of Cx can be incorporated into the bisexual population that provides the male, or whether the all-female population itself might undergo alterations as a result of continuously mating with other species.

In the Rio Mocorito, Cx is normally propagated by mating with males of *P. lucida,* a species so uniform morphologically as to offer no marker genes for tracing the paternal hereditary materials. This species, furthermore, is so similar to Cx that Miller (1960) referred to Cx as an all-female form of *lucida.* Detailed morphological analyses, however, reveal differences in the structure of the mouth, in the pigmentation (live specimens only) and in the structure of the genitalia, in the teeth, in the ova size, and in others, all of which remain consistent for Cx even after 10 generations of *lucida* matings. In view of this seemingly matroclinous mode of inheritance, the fate of the paternal chromosomes becomes the key to an understanding of the all-female mechanism. By selecting *P. latidens* as a mate for Cx, a great constellation of contrasting characters was made available, from which 6 were selected for detailed study in hybrid crosses with Cx. The following characteristics were found to differ markedly in all three forms, that is, Cx, *lucida,* and *latidens:* (1) genitalia; (2) lip shape; (3) structure of the dentary bones; (4) number, form, and size of the inner and outer teeth; (5) body pigments; and (6) ova size. Since expression of most of these characters, ova size being a possible exception, appears to be polygenic, marker genes, in a sense, are provided for a large portion if not all of the male's chromosomes.

The inheritance pattern of these characters in F_1 progeny of the Cx $\times$ *latidens* cross is by and large one of intermediacy; however, the form, count or measurement is usually somewhat closer to that of one parent than the other. The shape of the lips, the shape and size of the outer teeth, and the width of the inner teeth, for example, although intermediate, more nearly approach those of *latidens,* whereas the shape of the inner teeth and the ova size are the same as those of Cx. Judgment of bone shapes and pigment patterns is too subjective to enable more than a declaration of intermediacy. The inheritance patterns of the characters involved is not unexpected for traits influenced by multiple genes and the fact that they are not exactly intermediate might simply indicate unequal dosage effects from the different parents. The fact that the outer teeth in both the Cx $\times$ *latidens* cross and the F_1 $\times$ *latidens* backcross are fewer in number than in either parental form would at first seem contradictory to all that's reasonable. Once the composition and reproductive mechanisms of Cx are more adequately defined this matter will be accounted for. In any case, inheritance of the traits studied leaves no question that both parents contribute significantly to the genetic makeup of the F_1 offspring.

If inheritance now in the backcross matings is typical of what is true of other fish hybrids (Hubbs, 1955; Atz, 1962), one would expect the backcross progeny in the one direction to be more like *latidens* than the F_1, and in the other direction more like *lucida* but with some *latidens* traits persisting. This is not the case, however. When F_1 females were mated to *lucida* males, not a trace of *latidens* influence remained in the offspring—all were identical to Cx in every respect. It is apparent that none of the *latidens* chromosomes were transmitted through the

eggs: All were lost, presumably during oogenesis. There, furthermore, is no evidence that crossing-over introduces male genes into the Cx line. This means, then, the eggs produced by the F_1 female that united with the *lucida* sperm were purely matroclinous. It would follow that in the backcross of the Cx × *latidens*, F_1 to *latidens* this egg, too, would contain only the genetic elements of the mother; hence, the phenotypic backcross in reality is genetically identical to the initial Cx × *latidens* mating. This supposition proves to be true by the fact that the backcross progeny were identical to the F_1 progeny—no increase in *latidens* traits took place.

We deal thus with an isolating mechanism that is complete. There is little likelihood that wild populations of Cx are genetically altered by either *lucida* or *latidens* but that the male genome is only borrowed for the production of each generation. It is furthermore unlikely that Cx elements are incorporated in the gene pools of either bisexual form.

The frequency with which Cx and *latidens* hybridize in nature has yet to be determined. The matter is complicated by the fact that numerous spotted forms found in the Mocorito resemble the Cx × *latidens* hybrid but are not identical and seem to be the result of another hybrid phenomenon not yet resolved. There is, however, no question that the Cx × *latidens* cross does take place. From 9 pregnant wild Cx females separated from males at the time of their capture and later isolated in aquaria, 69 young were produced which upon reaching maturity were typically Cx and were obviously sired by wild *lucida* males. One of these females, however, in addition to 6 Cx-like young, produced a spotted offspring characteristic of the Cx × *latidens* F_1. This Cx female evidently had mated with both *lucida* and *latidens* males and produced progeny by each in a single brood.

In view of what is happening here genetically, a rather knotty problem of nomenclature arises. Normally one might assign to Cx a Latin name such as *Poeciliopsis prostituere* and when it hybridizes with *latidens*, expand the name to form *P. prostituere-latidens*; however, Cx is already a hybrid, with *lucida* as one of the parents. Since all of the paternal chromosomes of each generation are lost it is only the female genome that combines with *lucida* on the one hand and *latidens* on the other. Not only is the naming of a genome unprecedented but added to this is the fact that we do not even know what this genome looks like since the only observable morphological expression is as a hybrid in combination with *lucida* and occasionally *latidens*.

The earlier view (Schultz, 1961) that this mysterious genome itself has had a hybrid origin seems no less likely. It may in fact explain why the Cx × *latidens* offspring have fewer outer teeth than any of the parental forms involved in the matings. If one of the species of the initial cross that resulted in the all-female genome had a low tooth count, then the genes for tooth expression carried by this genome would call for a low number of outer teeth. This genome, therefore, in combination with *lucida*, which has many teeth (29.07), results in Cx, a form with a moderate number of teeth (27.00). If the tooth count of the all-female genome is less than that of *latidens* (24.15), then in combination with this species, intermediate expression results in a tooth count lower (21.47) than either of the phenotypic parents (Cx or *latidens*).

If such a mechanism is operational as far as the outer teeth are concerned, there

is no reason to believe that it is not also operational in the formation of other characters as well. The fact that many of the characters of the Cx × *latidens* F_1 progeny were not exactly intermediate between the parental forms, as Hubbs (1955) and others found in hybrids, but were closer to *latidens,* suggests that this all-female genome carries traits rather similar to *latidens.* The production of some Cx × *latidens* F_1 males (6.7%) indicates that the strength of the sex-determining genes of the all-female genome is somewhat more comparable to that of *latidens* than to that of *lucida* since all progeny from Cx × *lucida* matings are females. The implication here does not refer to a likelihood that *latidens* participated in the hybrid synthesis of the all-female genome. It seems more plausible that a species related to *latidens* was involved. It is further expected that when this species is found and mated to Cx, a breakdown of the all-female mechanism, complete with the production of fertile males, will occur along with the restoration of random assortment during meiosis.

Collections from specific localities in the Rio Mocorito contain, in addition to the above forms, *Poeciliopsis viriosa* Miller. The evidence from preserved material suggests that this species has not only hybridized with Cx in nature but that the F_1 progeny have mated with both *lucida* and *viriosa,* producing backcross progeny recognizable as such. Experimental studies directed toward a verification of the morphological determinations are currently in progress. It is possible that these studies will provide a key to the origin of the all-female genome and ultimately lead to its synthesis in the laboratory.

Summary

1. Hybridization experiments between an all-female fish, *Poeciliopsis* "Cx," and the males of two bisexual species, *P. lucida* and *P. latidens,* have provided genetic evidence that hereditary materials of both the mother and the father combine to form the F_1 progeny. The entire male genome, however, appears to be simply "on loan" for each generation. None of the male's chromosomes, apparently, are incorporated in the ova; thus, the integrity of the maternal line is maintained, even though the all-female form is forced to rely on sperm from other species to maintain itself.

2. This mode of reproduction provides an isolating mechanism as complete as that of parthenogenesis or gynogenesis.

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